PATENT COOPERATION TREATY

PCT

RECD 11 APR 2001
WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
C 2260 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/00597	26/01/2000	27/01/1999
International Patent Classification (IPC) or national classification and IPC A61K9/127		
Applicant IDEA AG		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 19 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
24/08/2000	04.04.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Favre, N Telephone No. +49 89 2399 7363

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/00597

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-28,32-52	as originally filed			
29,29a,30,30a, 31	as received on	02/11/2000	with letter of	08/05/2000

Claims, No.:

1-36	as originally filed			

Drawings, sheets:

1/14-14/14	as received on	02/11/2000	with letter of	08/05/2000

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 25-35, with respect to industrial applicability.

because:

   ☒ the said international application, or the said claims Nos. 25-35 relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-35
	No:	Claims	36
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-36
Industrial applicability (IA)	Yes:	Claims	1-24 and 36
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 25-35 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. For the assessment of the present claims 25-35 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

2. Document D1 (Vaccine Research, 1995, 4(3):145-164) describes a **transdermal vaccine** (cf. abstract) using specially optimised ultradeformable agent carriers, named transfersomes™, in combination with different adjuvants. Document D1 shows that the therein described composition elicits a specific immune response in a murine experimental model, when applied transdermally.
As far as it can be understood (see Item VIII) and according to the applicant's arguments, the subject-matter of independent claim 1 differs from the disclosure

of D1 in that a compound which specifically releases or induces cytokine or anti-cytokine activity, or exerts such an activity itself (see claim 1(b)) is present in the claimed composition (see claim 8 for examples of such compounds).
According to the applicant this feature allows the successful induction of a medically useful transdermal immune response (see also page 7, lines 13-16 of the description).
However, the sole example using the compounds as required by claim 1 (b) which has provided in the application as filed is the set of experiments illustrated in Figure 9. As can be read in the legend of said Figure 9, **no protection was observed in these experiments.**
Therefore, the composition defined in independent claim 1 fails to solve the above stated technical problem and hence cannot be considered as being inventive in the sense of Article 33(3) PCT.

2.1 Dependent claims 2-22 which characterise further embodiments of claim 1, claims 23 and 24 which define kits comprising the vaccine composition of claim 1, and claims 25-35 which define different uses of the vaccine composition of claim 1 for the generation of a protective immune response do not appear to introduce subject-matter which would render the subject-matter of said claims inventive in view of the disclosures of D1.
Claims 2-35 thus do not fulfill the requirements of Article 33(3) PCT.

2.2 Claim 36 refers to the use of any **individual** compound as defined in any of the preceding claims for the preparation of a vaccine composition which would induce any immune response. Among **many** other examples, claim 36 combined with claim 11 includes any known and unknown vaccine.
Claim 36 is therefore not novel in the sense of Article 33(2) PCT.

Re Item VIII

Certain observations on the international application

1. Claim 1 does not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not defined. The claim attempts to define the subject-matter in terms of the result to be achieved.

1.1 Moreover, claim 1 is not supported by the description as required by Article 6 PCT, as its scope is much broader than justified by the description and drawings, in which only one embodiment which allows the performance of the claimed invention is disclosed, i.e. the use of transfersomes™. Furthermore, some of the conventional lipid vesicles described in the comparative examples also fall within the broad wording of the claim. It is generally accepted that the disclosure of one way of performing an invention is only sufficient if it allows the invention to be performed in the **whole range claimed** rather than only some members of the claimed class to be obtained (see also Item V).

1.2 In addition, as sufficiency of disclosure thus presupposes that the skilled person is able to obtain substantially all embodiments falling within the ambit of the claims, the present application does not meet the requirements of Article 5 PCT (see also Item V).

2. The extensive use in the claims of the expressions "one or more", "preferably", "and/or", "in particular", "such as", "like", "etc.", "often" and of similar formulations renders the determination of the exact nature of the protection sought nearly impossible. Therefore, claims 1-36 lack clarity in the sense of Article 6 PCT.

repeated immunogen administration is advocated to maximize the final effect of a therapeutic vaccination. It is proposed to use between 2 and 10, often between 2 and 7, more typically up to 5 and most preferred up to 3 immunizations, when a non-allergenic antigen is used, or such a number of times, in the case of allergens, as is required either to achieve the desired immuno-tolerance, determined as described above or another suitable assessment method, or else to deem the effort as having failed. The time interval between subsequent vaccinations should preferably be between 2 weeks and 5 years, often between 1 month and up to 3 years, more frequently between 2 months and 1.5 years, when a subject is being immunized for the first time. Rodents, such as mice and rabbits are advantageously immunized in 2 weeks interval, primates, e.g., monkeys and often humans, need a booster vaccination in 3-6 months interval.

In a preferred embodiment of the method according to the present invention the flux of penetrants that carry an immunogen through the various pores in a well-defined barrier is determined as a function of a suitable driving force or a pressure acting across the barrier and the data are then conveniently described by a characteristic curve which, in turn, is employed to optimize the formulation or application further.

The invention finally relates to the use of the transdermal carrier, the compound which specifically releases or specifically induces cytokine or anti-cytokine activity or exerts such an activity, the antigen or allergen, and optionally an extract or a compound from a microorganism or a fragment or a derivative thereof, and/or a low molecular weight chemical irritant as defined hereinbefore for the preparation of a vaccine for inducing a protective or tolerogenic immune response.

~~The figures show:~~

Figure 1 gives the data on survival of animals immunized epicutaneously with mixed micelles or Transfersomes loaded with TT, to illustrate aggregate size (stability) effect; ~~since the over-destabilized Transfersomes normally disintegrate into the mixed lipid micelles.~~

The figures show:

Figure 1: Mixed micelles versus Transfersomes. The figure gives the data on survival of animals immunised epicutaneously with mixed micelles or Transfersomes loaded with purified TT, to illustrate aggregate size (stability) effect, since the over-destabilised Transfersomes normally disintegrate into the mixed lipid micelles.

Figure 2: Liposomes versus Transfersomes. A comparison is made between the immune response to conventional lipid vesicles (liposomes) and ultradeformable lipid vesicles (Transfersomes) carrying purified TT and applied on the skin. The information on corresponding specific antibody concentrations in serum (expressed as absorbance) is given in the upper panel.

Figure 3: Antigen dose effect. The figure illustrates the effect of increasing antigen dose on the outcome of epicutaneous immunisation by means of Transfersomes from SPC:NaChol (3.75:1) loaded with antigen and monophosphoryl lipid A (LA).The results are expressed as absorbance change, antibody titre, or animal survival, together with the corresponding specific antibody isotyping data. Antigen doses were 10, 20, 40 and 80 μg. 6 animals per each group except for No Ag (4 animals) were used.

Figure 4: Antigen purity effect. The figure highlights the effect of antigen purity on the result of epicutaneous immunisation with 80 μg tetanus toxoid and monophosphoryl lipid A (LA) in Transfersomes from SPC:NaCh (3.75:1), including information on time dependence of animal survival. All data were obtained after the 2nd boost + 7 days.

Figure 5: Epicutaneous versus subcutaneous immunization. The figure compares the outcome of repeated invasive (subcutaneous) and non-invasive (epicutaneous) immunisation by means of TT in Transfersomes, including animal

survival, serum concentration (in terms of absorbance), specific antibody titre, and antibody distribution pattern values.

Figure 6: Pre-injection effect. The figure illustrates the effect of skin pre-treatment (non-specific challenge) on the immune response following Transfersome (SPC:Tw-80 1:1) mediated TT (40 μg) delivery across the skin. Mice in the preinjection groups were injected 24 hours before the application of 40 μg antigen. 0.1 ml each of saline (pre-S), 10% SPC:NaCh 4.5:1 empty Transfersomes (Pre-empty Tfs), and incomplete Freund's adjuvant were used for pre-injection. All mice in this experiment were challenged with 50 times LD50 dose of toxin 7 days after the second boost. It means (ec) epicutaneous, (sc) subcutaneous, and (Tfs) Transfersomes.

Figure 7: Adjuvant effect: for example monophosphoryl lipid A. The figure focuses on adjuvant effect of a relatively low-molecular weight immuno-stimulator, monophosphoryl Lipid A (LA), delivered across intact skin together with TT in Transfersomes.

Figure 8: Adjuvant effect: for example cytokine IL-12. The figure demonstrates the immuno-adjuvancy of a cytokine, interleukin-12 (IL-12) transported across the skin (ec) together with TT by means of Transfersomes from SPC:NaCh.

Figure 9: Immunomodulant effect, for example cytokines. The figure deals with the immuno-modulation by various cytokines of the murine response against impure tetanus toxoid (TT) antigen delivered in Transfersomes non-invasively through the skin. Serum was collected for the assay on the 7th day after 2nd boost. No protection was observed in any of the groups.

Figure 10: Immunoadjuvant effect: for example cholera toxin (CT). The figure presents experimental evidence for the immune response stimulation of mice treated on the skin by pure tetanus toxoid (TT) in Transfersomes (SPC:NaCh 3.75:1), when the carriers also include 10 μg cholera toxin (CT) to support the

specific antibody production, and thus animal protection against an otherwise lethal challenge by the tetanus toxin. 4-6 animals per group were used. The asterisc indicates 1 paralyzed mouse out of 4.

Figure 11 Adjuvant effect: for example heat labile toxin (HLT) from E.coli. The figure illustrates the use of heat labile toxin from E. coli as an immuno-adjuvant.

Figure 12: Histamine effect: on anti-tetanus titer and survival after immunization with Transfersomes on the skin. The figure illustrates the immuno-modulating effect of local skin pre-treatment with histamine in combination with transdermal antigen application with Transfersomes.

Figure 13: Subcutaneous priming: effect on anti-tetanus titer and survival after epicutaneous boosts. The figure demonstrates the effect of subcutaneous priming on anti-tetanus titer and on the survival of epicutaneously vaccinated hosts.

Figure 14: Bi-valent vaccines: Anti-Tetanus and anti-Cholera response to the administration of both antigens together in Transfersomes on the skin. The figure shows the effect of bi-valent vaccination with Tetanus Toxoid nad Cholera Toxin used as antigens.

~~Figure 10 presents experimental evidence for the immune response stimulation of~~ mice treated on the skin by TT in Transfersomes, when the carriers also include cholera toxin (CT) to support the specific antibody production, and thus animal protection against an otherwise lethal challenge by the tetanus toxin.

Figure 11 illustrates the use of heat labile toxin from E. coli as an immuno-adjuvant.

Figure 12 illustrates the immuno-modulating effect of local skin pre-treatment with histamine in combination with transdermal antigen application with Transfersomes.

Figure 13 demonstrates the effect of subcutaneous priming on anti-tetanus titer and on the survival of epicutaneously vaccinated hosts.

Figure 14 show the effect of bi-valent vaccination with Tetanus Toxoid and Cholera ~~Toxin used as antigens.~~

The examples illustrate but do not define the limits of the invention.

General experimental set-up and sample preparation

Mice of Swiss albino strain (18-20 g) were obtained from The National Institute of Nutrition (Hyderabad, India). They were 8 to 12 weeks old at the time of first immunization and were normally kept in suspension cages in groups of 4 to 6. The animals had free access to standard chow and water. One day prior to an immunization, the application area on murine back was shaved carefully. The antigen was administered with a high precision pipette on the skin surface and left to dry out partially. To prevent immunogen abrasion, the animals were transferred into individual cages in which they were kept for 18 hours following each epicutaneous material administration.

General anesthesia was used to keep the test animals stress free and quiet during manipulations, including immunization. An injection of a mixture of Ketavet and Rompun (0.3 mL per mouse of an isotonic NaCl solution containing 0.0071 % Rompun

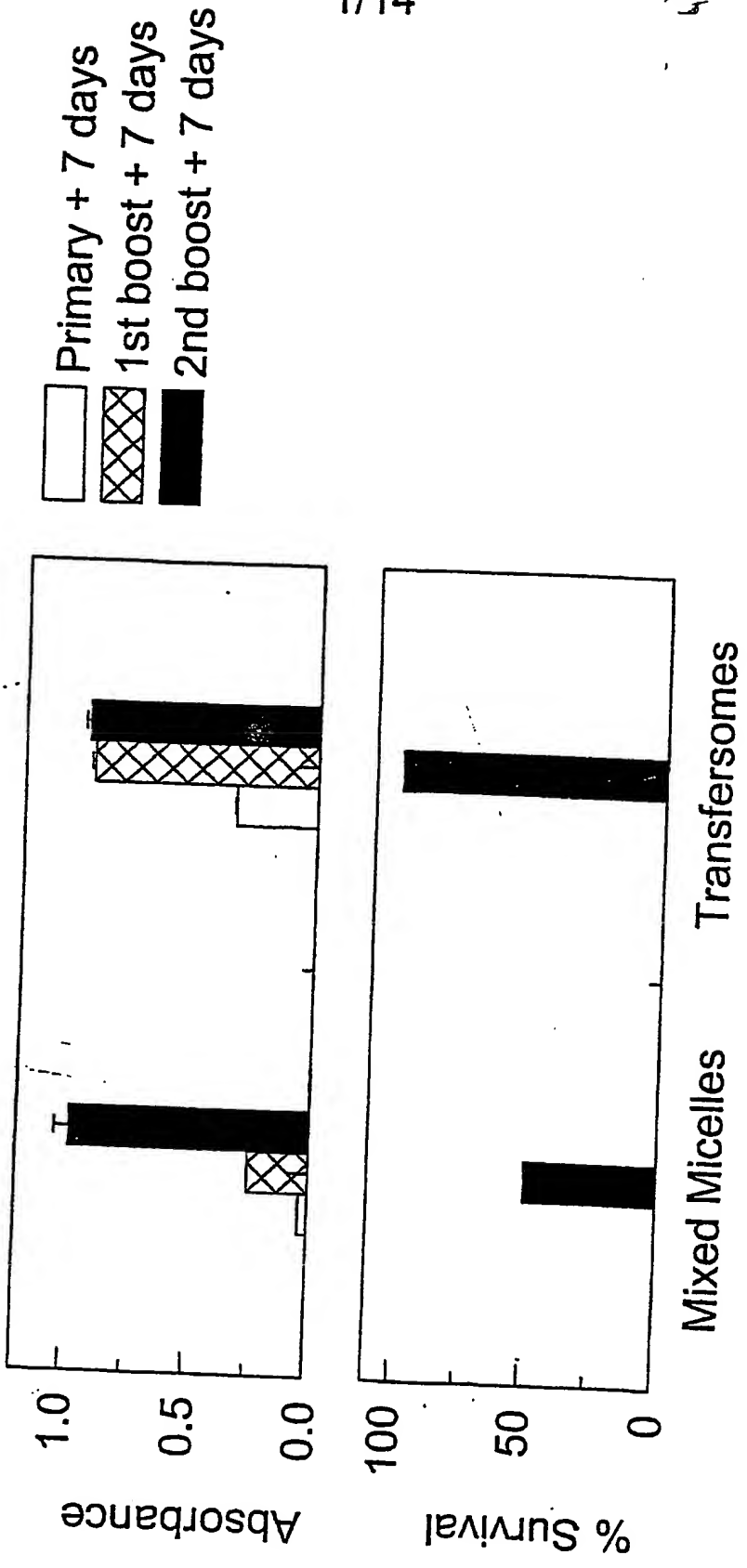

Primary + 7 days
1st boost + 7 days
2nd boost + 7 days
Absorbance
1.0
0.5
0.0
% Survival
100
50
0
Mixed Micelles
Transfersomes

Fig. 1

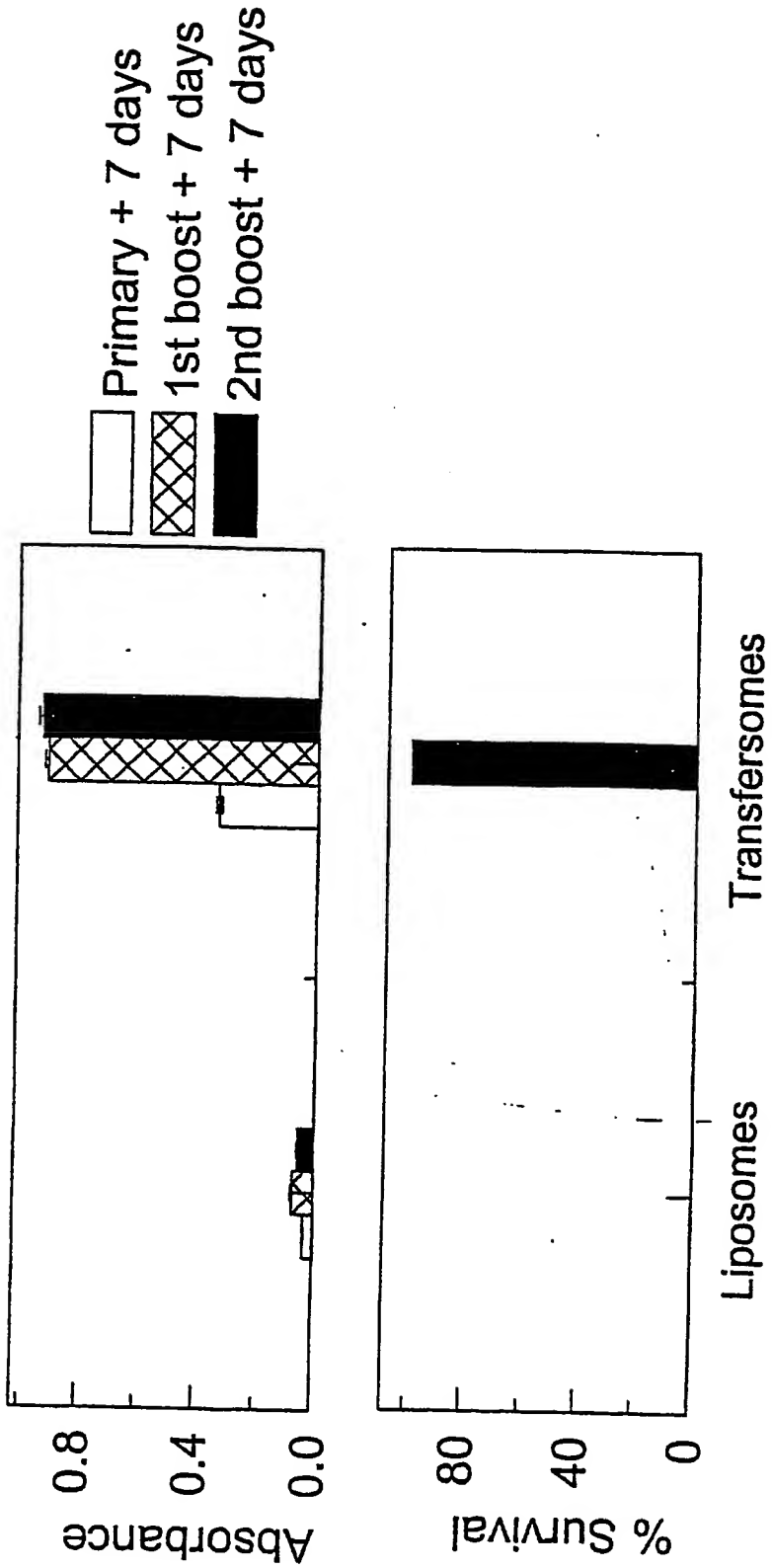

Primary + 7 days
1st boost + 7 days
2nd boost + 7 days
Absorbance
0.0
0.4
0.8
% Survival
0
40
80
Liposomes
Transfersomes

Fig. 2

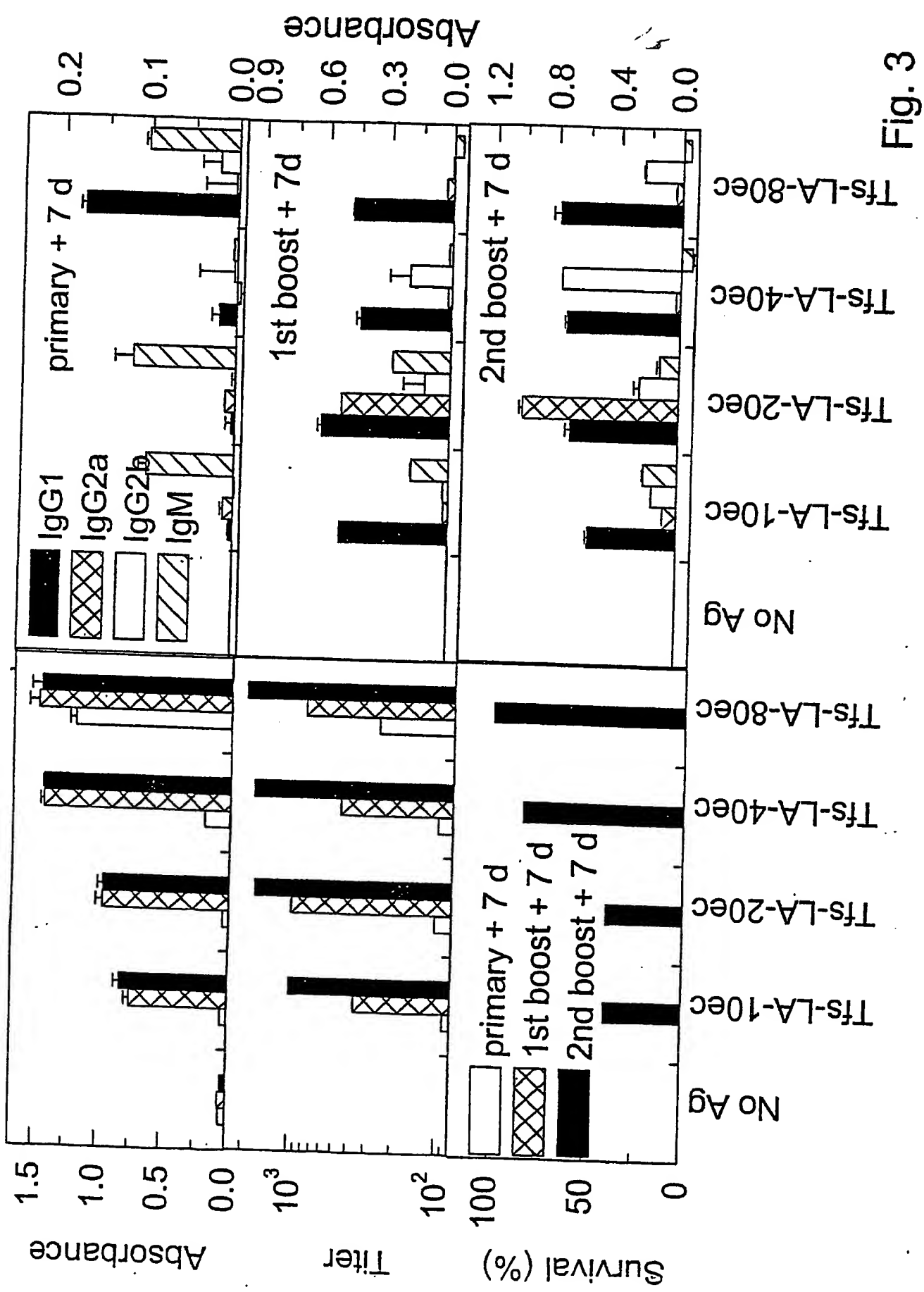

Absorbance
Titer
Survival (%)
primary + 7 d
1st boost + 7d
2nd boost + 7 d
IgG1
IgG2a
IgG2b
IgM
No Ag
Tfs-LA-10ec
Tfs-LA-20ec
Tfs-LA-40ec
Tfs-LA-80ec

Fig. 3

Impure toxoid
Partially purified toxoid
Pure toxoid
Survival (%)
Titer
Absorbance
epicutaneous
n=6
Impure toxoid-80µg
Partially purified toxoid-80µg+LA
Pure toxoid-80µg+LA
IgG1
IgG2a
IgG2b
IgG3
IgA
IgM
Absorbance
Impure toxoid 80 µg
Partially purified toxoid 80 µg
Pure toxoid 80 µg
Survival (%)
Time (hours)

Fig. 4

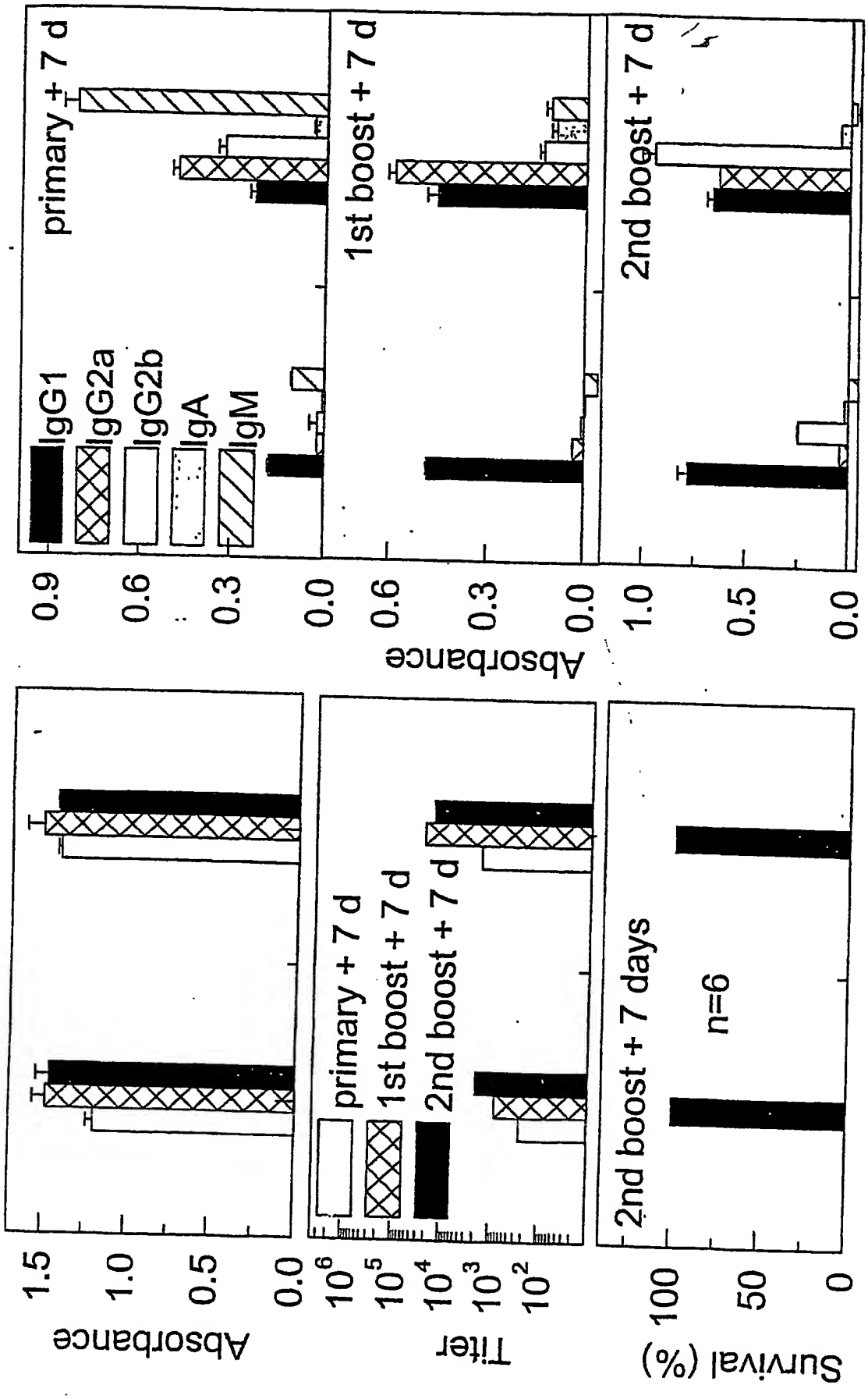

primary + 7 d
1st boost + 7 d
2nd boost + 7 d
IgG1
IgG2a
IgG2b
IgA
IgM
Absorbance
0.9
0.6
0.3
0.0
1.0
0.5
epicutaneous subcutaneous
pure tetanus toxoid
Titer
10^6
10^5
10^4
10^3
10^2
2nd boost + 7 days
n=6
Survival (%)
100
50
0
1.5

Fig. 5

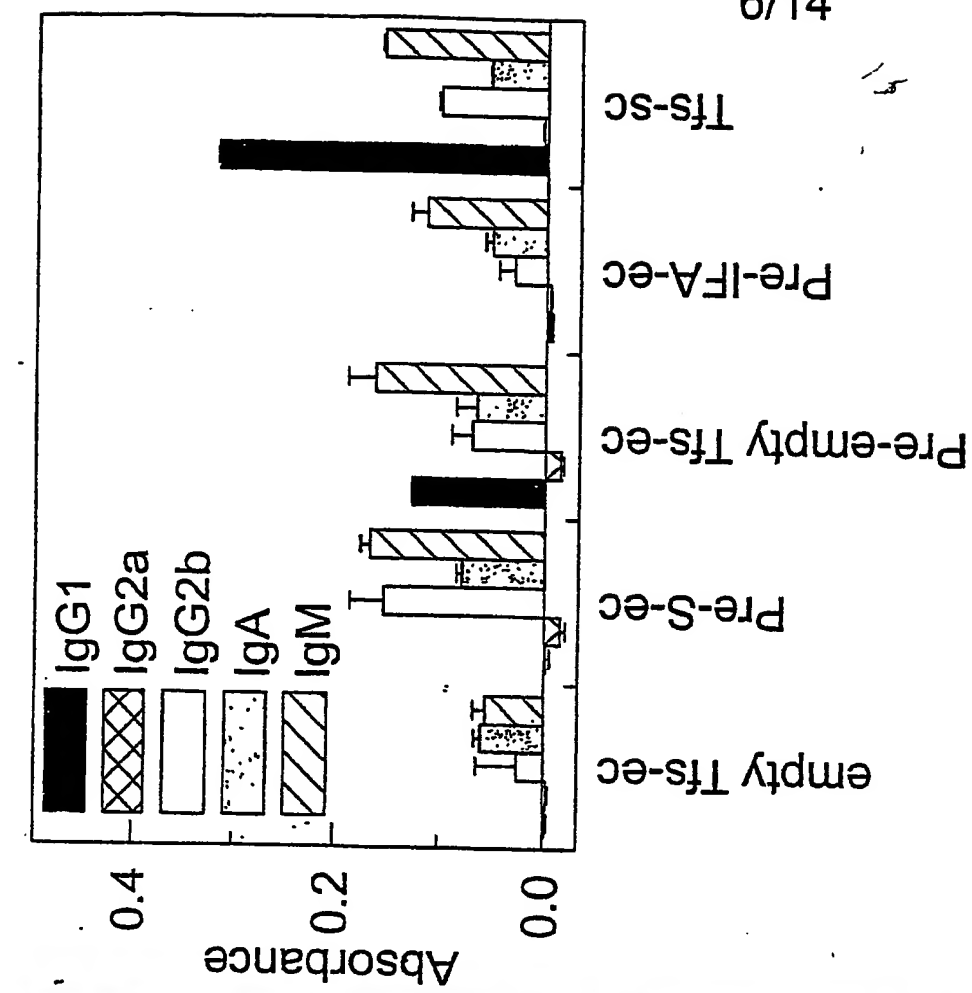

Absorbance
0.0
0.2
0.4
IgG1
IgG2a
IgG2b
IgA
IgM
empty Tfs-ec
Pre-S-ec
Pre-empty Tfs-ec
Pre-IFA-ec
Tfs-sc
Survival (%)
0
25
50
Titer
10^1
10^2
10^3
primary+7 d
1st boost+7 d
2nd boost+7 d
Absorbance
0.0
0.6
1.2
n=6
empty Tfs-ec
Pre-S-ec
Pre-empty Tfs-ec
Pre-IFA-ec
Tfs-sc

Fig. 6

IgG1
IgG2a
IgG2b
IgA
IgM
primary + 7 d
1st boost + 7 d
2nd boost + 7 d
Absorbance
0.2
0.1
0.0
0.8
0.4
0.0
1.2
0.8
0.4
0.0
Tfs-40-ec
Tfs-LA40-ec
Tfs-80-ec
Tfs-LA80-ec
primary + 7 d
1st boost + 7 d
2nd boost + 7 d
Absorbance
2.0
1.6
1.2
0.8
0.4
0.0
Titer
10^3
Survival (%)
100
50
0
* partially paralyzed but alive
Tfs-40-ec
Tfs-LA40-ec
Tfs-80-ec
Tfs-LA80-ec

Fig. 7

Survival (%)
100
50
0
Titer
10^3
10^2
Absorbance
1.2
0.8
0.4
0.0
primary + 7 d
1st boost + 7 d
2nd boost + 7 d
Tfs-80-ec
Tfs-LA-IL-12-80-ec
Absorbance
0.4
0.2
0.0
2nd boost + 7 d
Tfs-80-ec
Tfs-IL-12-80-ec
IgG1
IgG2a
IgG2b
IgA
IgM

Fig. 8

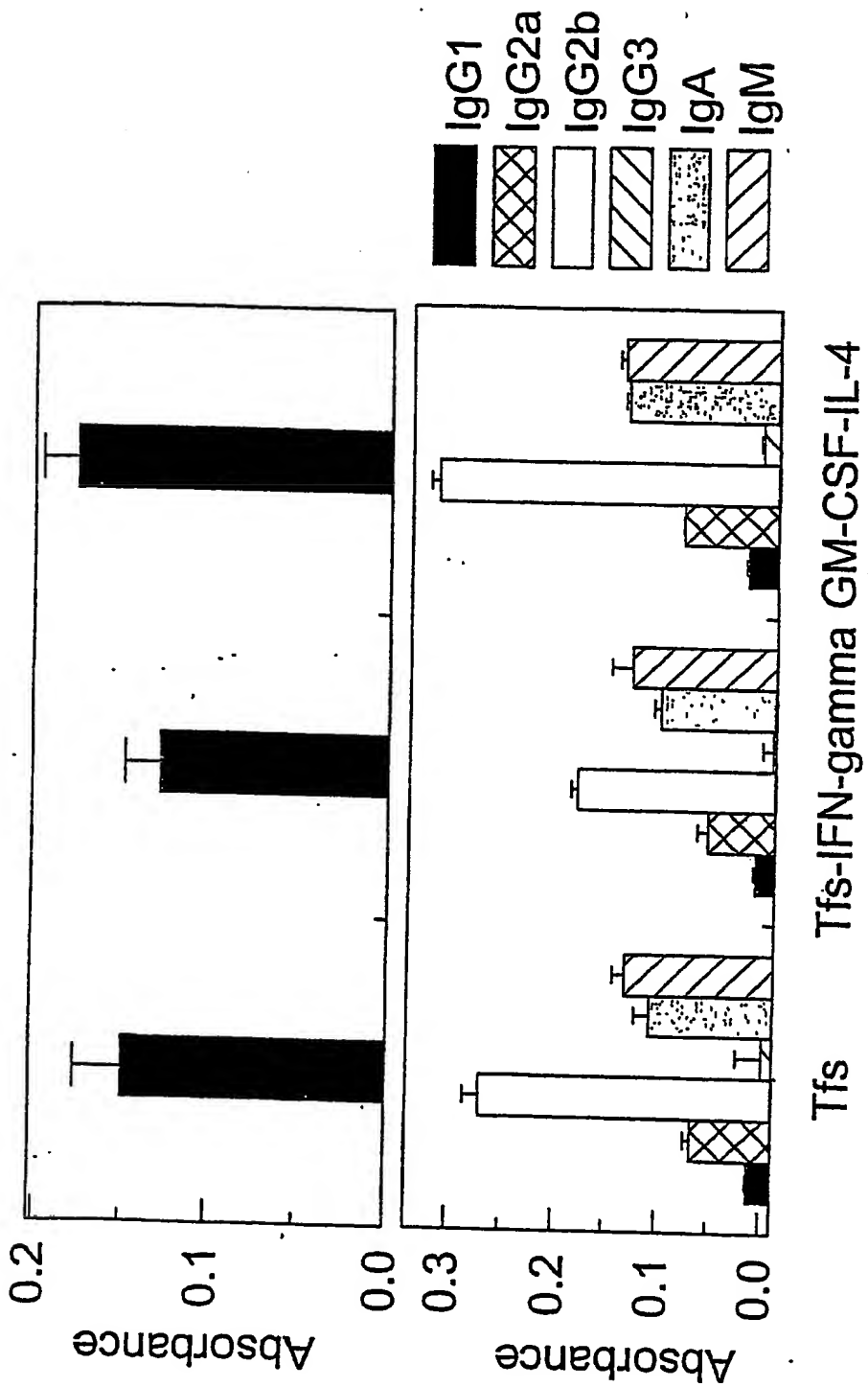

IgG1
IgG2a
IgG2b
IgG3
IgA
IgM
Absorbance
0.0
0.1
0.2
0.3
Tfs
Tfs-IFN-gamma GM-CSF-IL-4

Fig. 9

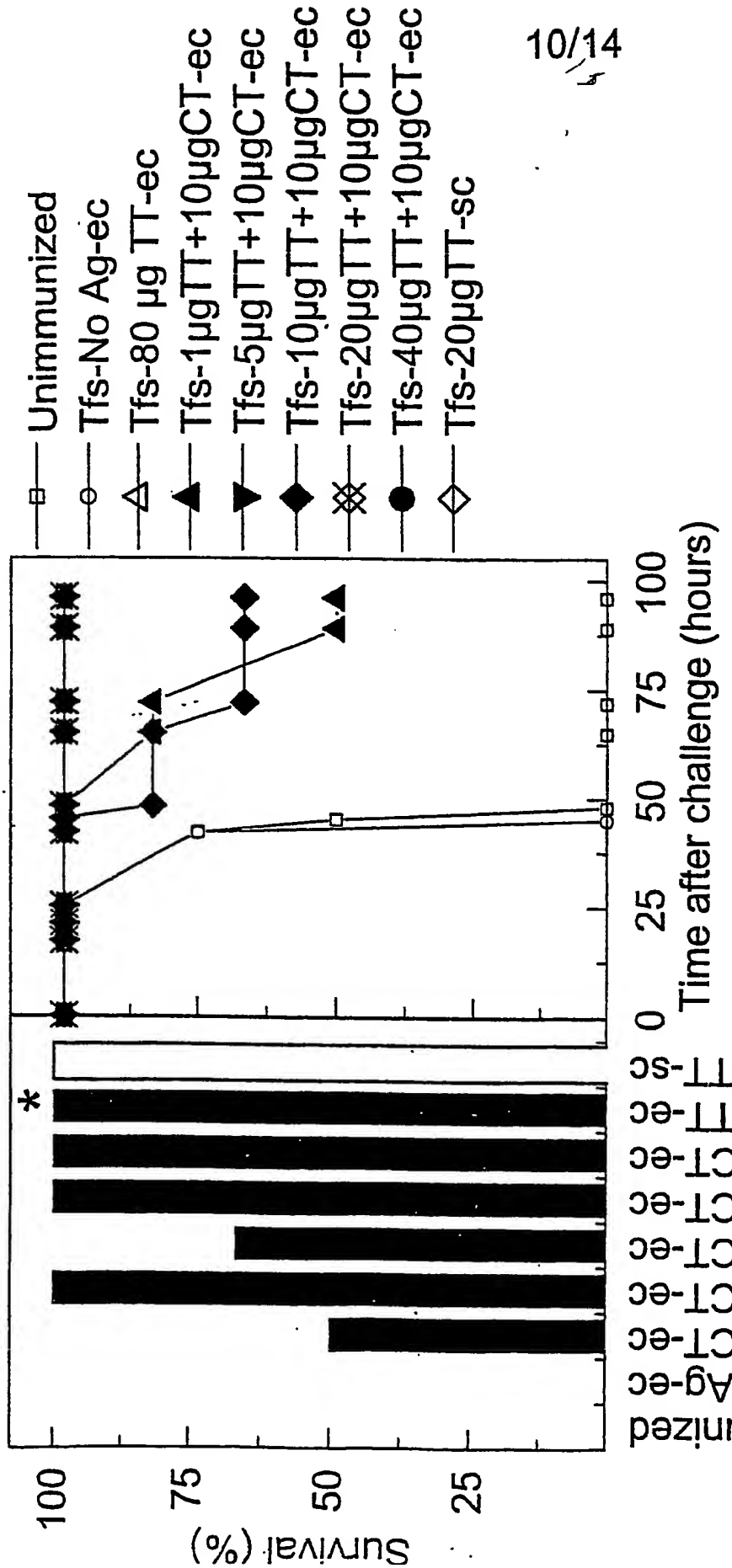
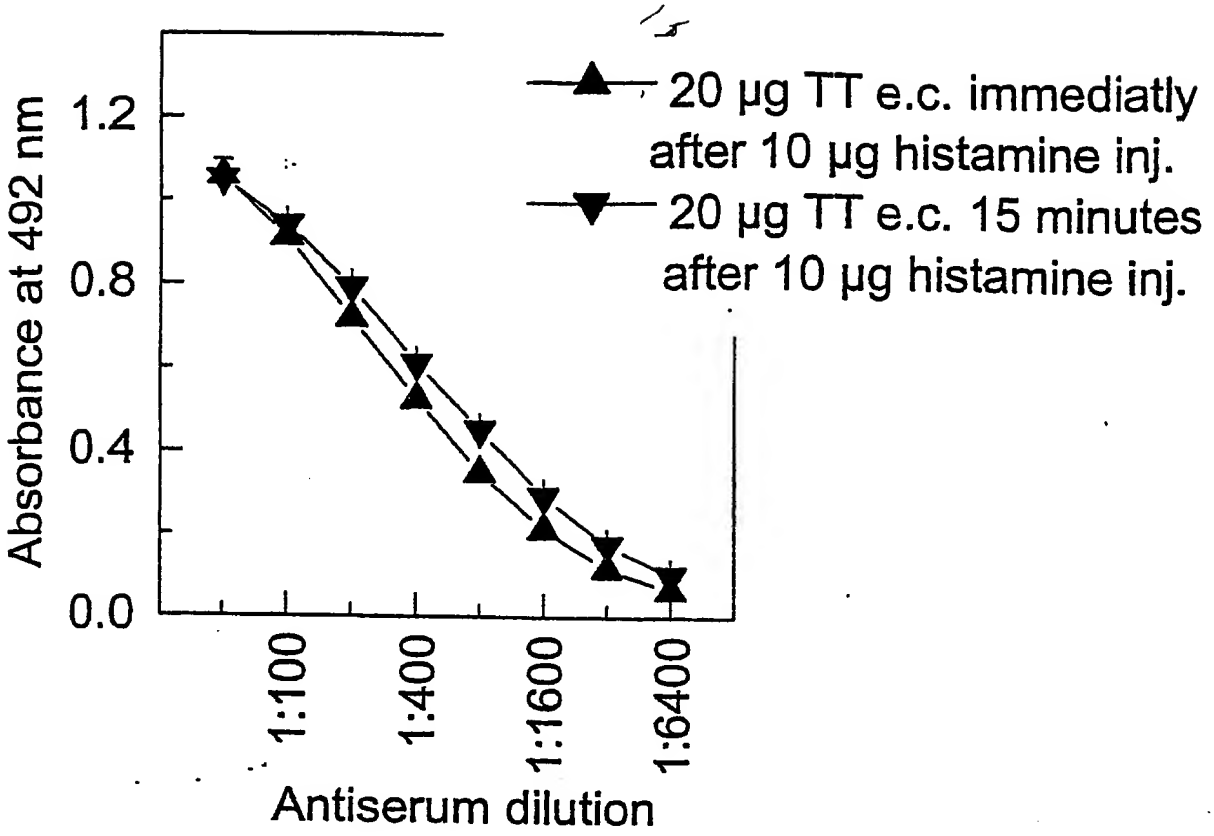

Survival (%)
100
75
50
25
0
25
50
75
100
Time after challenge (hours)
Unimmunized
Tfs-No Ag-ec
Tfs-1µgTT+CT-ec
Tfs-5µgTT+CT-ec
Tfs-10µgTT+CT-ec
Tfs-20µgTT+CT-ec
Tfs-40µgTT+CT-ec
Tfs-80µgTT-ec
Tfs-20µgTT-sc
*
Unimmunized
Tfs-No Ag-ec
Tfs-80 µg TT-ec
Tfs-1µgTT+10µgCT-ec
Tfs-5µgTT+10µgCT-ec
Tfs-10µgTT+10µgCT-ec
Tfs-20µgTT+10µgCT-ec
Tfs-40µgTT+10µgCT-ec
Tfs-20µgTT-sc

Fig. 10

Absorbance at 492 nm
0.0
0.4
0.8
1.2
1:100
1:400
1:1600
1:6400
Antiserum dilution
20µgTT+1µgHLT e.c.
20µgTT+2µgHLT e.c.
0.5µgTT s.c.
% survival
0
25
50
75
100
20µgTT/1µgHLT e.c.
20µgTT/2µgHLTe.c.
0.5µgTTs.c.

Fig. 11


20 µg TT e.c. immediatly after 10 µg histamine inj.
20 µg TT e.c. 15 minutes after 10 µg histamine inj.
Absorbance at 492 nm
1.2
0.8
0.4
0.0
1:100
1:400
1:1600
1:6400
Antiserum dilution

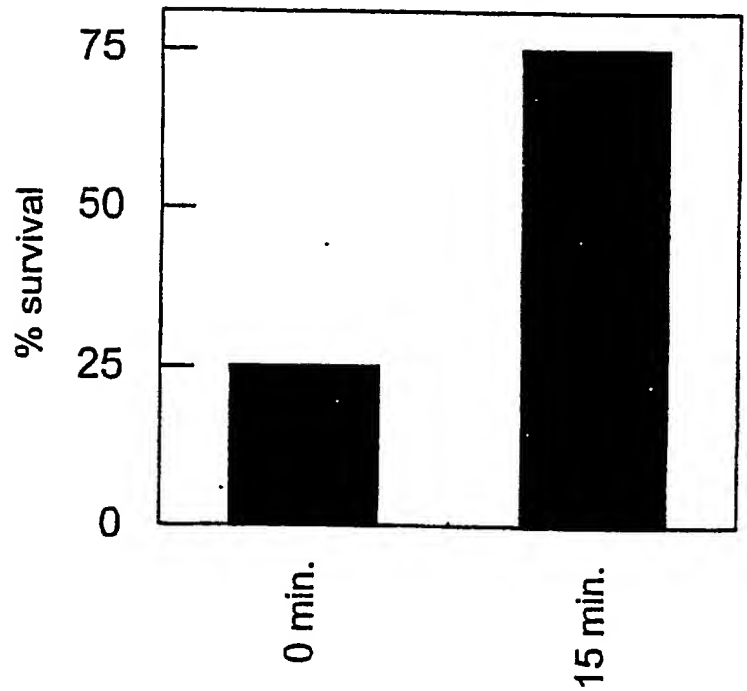

% survival
75
50
25
0
0 min.
15 min.

Fig. 12

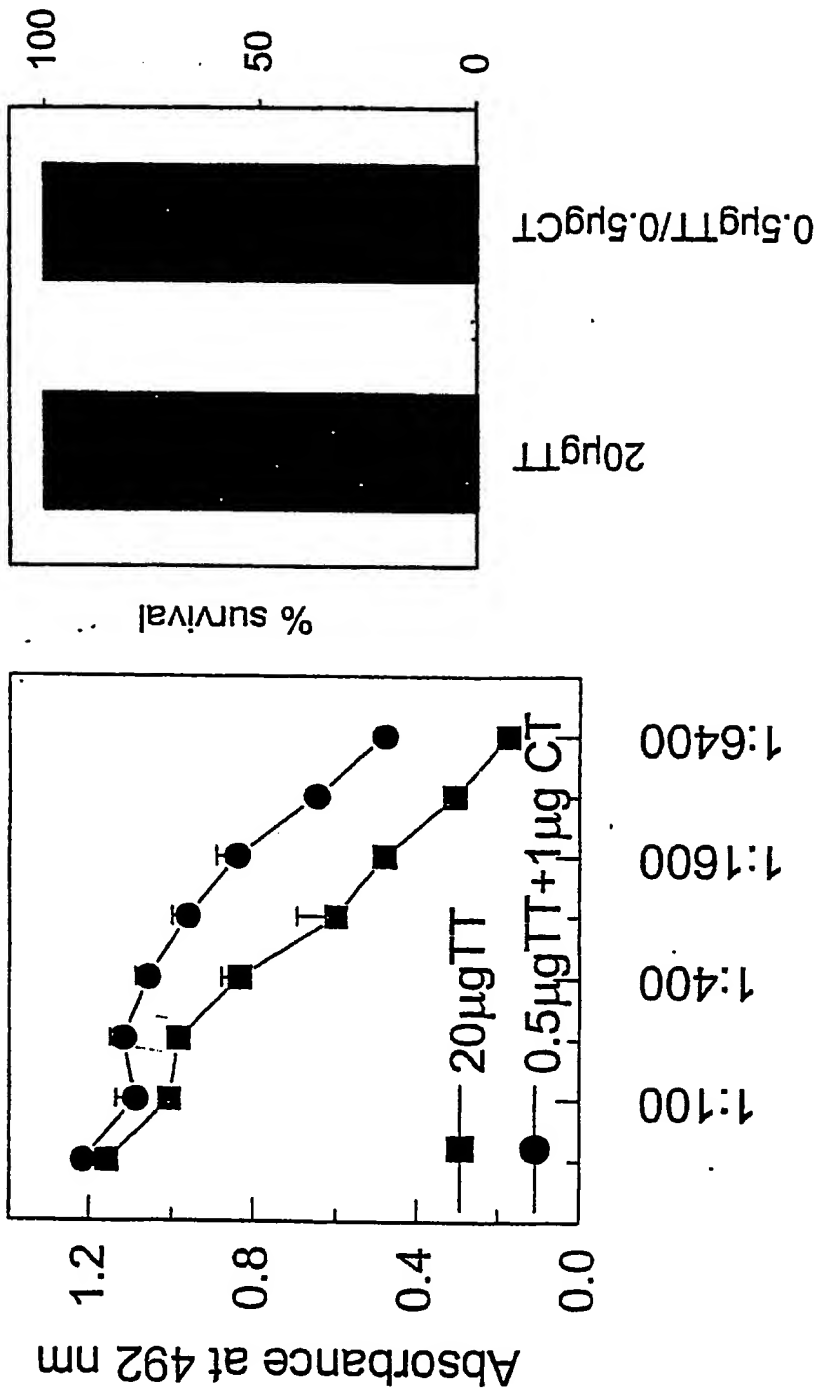

100
50
0
% survival
20µgTT
0.5µgTT/0.5µgCT
Absorbance at 492 nm
1.2
0.8
0.4
0.0
20µgTT
0.5µgTT+1µg CT
1:100
1:400
1:1600
1:6400
Antiserum dilution

Fig. 13

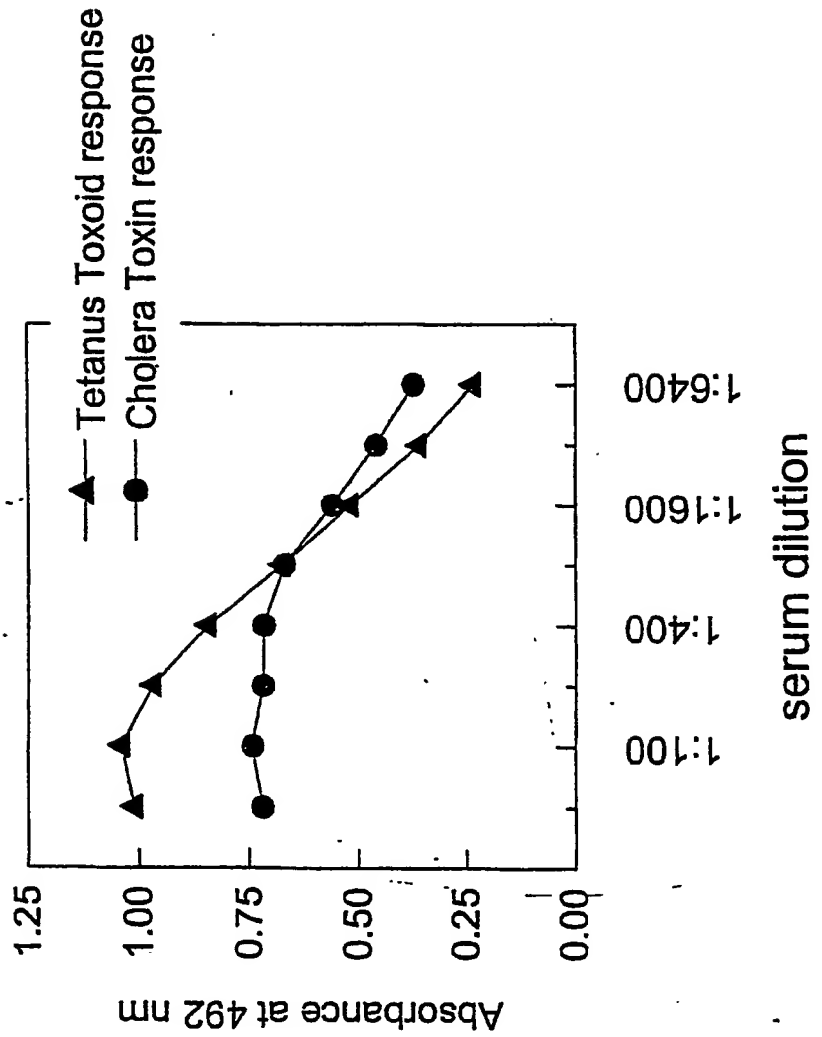

Tetanus Toxoid response
Cholera Toxin response
Absorbance at 492 nm
1.25
1.00
0.75
0.50
0.25
0.00
1:100
1:400
1:1600
1:6400
serum dilution

Fig. 14